10/30/98

Nonclinical Pharmacology and Toxicology Review of BLA 98-0286

Sponsor: Immunex

Product: Enbrel, TNF:Fc, 10 and 25 mg

Indication: Treatment of Rheumatoid Arthritis, sc administration

Introduction

rTNF:Fc is a fusion protein of the human p75 TNF receptor attached to the Fc portion of human IgG1. The fusion protein is produced in Chinese hamster ovary cells.

The anticipated therapeutic dosing regimen for rheumatoid arthritis is twice weekly sc injections of 25 mg. The dose of 25 mg in a 50 kg individual has an associated systemic exposure measured as AUC of 300 ug-hr/ml.

The protein is thought to act as a competitive inhibitor of TNF thereby inhibiting its binding to cell surfaces. Lymphotoxin α (LT) also binds to fusion protein and inhibition of LT binding may also contribute to the therapeutic activity of the product. TNF:Fc exhibits an affinity of 10^{10} M-1 which approximates the affinity of TNF for its cell surface receptor. Various studies demonstrate that TNF:Fc neutralizes the lethal biological activity of exogenously administered TNF in vivo . In vivo effects ameliorated by the fusion protein include induction of a shock-like syndrome. A list of pharmacology studies which were performed in support of TNF:Fc are attached as appendix A. Studies are grouped under mechanism of action, primary pharmacology (of direct interest to the clinical indication), secondary pharmacology, and pharmacologic interactions. The list of studies was supplied by the sponsor. The Fc portion of the molecule is not likely to participate in determining the pharmacological activity of the fusion protein. Although biologically active, the Fc portion's effects are inhibited by the far greater concentration of IgG molecules normally found in vivo. The fusion protein was found to be negative in a direct complement-mediated cytolysis and complement fixation assay.

Pharmacology

TNF:Fc was found to have activity in animal models of disease (lung inflammation model due to intranasal administration of actinomycete to mice and endotoxin challenge model in mice). Blood levels of TNF were reduced in the presence of the fusion protein, a finding consistent with the known, primary mechanism of action. The fusion protein also was found to reduce the biological activity of TNF in solid organ transplant patients given OKT3 for graft rejection. Serum concentrations of TNF were found to be elevated in the clinical study as the antigen recognized in the ELISA assayed was likely to have bound both the fusion protein and the combined product of the fusion protein and TNF (similar results were observed in comparable studies).

Animal models of autoimmunity and inflammation including arthritis were used to demonstrate the activity of TNF:Fc. The collagen induced arthritis model in mice was used in 2 mouse strains with three sources of collagen (bovine, porcine, and chicken) to demonstrate biological activity. In the bovine collagen induced model daily ip administration (50 ug/mouse) of the fusion protein reduced the incidence of disease (joint inflammation and tissue degradation) to 28% in comparison to 86% in saline treated controls. When treatment was delayed until the onset of disease, a less severe arthritis in mice given albumin was observed. The effects of TNF:Fc were dose dependent. TNFR:Fc was also effective in a rat model of antigen induced arthritis. In this model decreased joint swelling and less joint damage was observed in animals given an intra-articular injection of the TNF binding protein before or after antigen challenge (methylated BSA).

In addition to the effects in animal models of arthritis, TNFR:Fc was found to have activity in other animal models of disease. The fusion protein was effective in off-setting models of disease in the following types of studies: induction of wasting (cachexia), lung inflammation, allograft refection and vascular injury, autoimmune disease - multiple sclerosis (experimental allergic encephalomyelitis, chronic relapsing model induced by myelin proteolipid and *Mycobacterium tuberculosis*), cardiovascular dysfunction due to inflammation, and sepsis. In an animal model of infectious disease, TNFR:Fc had a detrimental effect. In a murine model of infection due to cytomegalovirus (MCMV), TNFR:Fc increased viral load and the number of infected liver cells.

Nonclinical ADME and Pharmacokinetics

A tissue distribution study of 125I-TNFR:Fc was conducted using mice (single iv or sc injection to BALB/c mice, N=3/time point, total N=27). Results of the study demonstrated that TNFR:Fc was distributed to all tissues examined (kidney, liver, lung, heart and spleen) with tissue concentration being always less than blood concentrations. After iv dosing, the spleen contained the highest concentration at 30 minutes for any of the solid organs examined for any time examined and after sc injection the lung contained the highest level at approximately 1 hour.

The pharmacokinetics of TNFR:Fc was investigated after various routes of administration (iv, sc) in different species of animals (mouse, cynomolgus monkey, Sprague-Dawley and CD VAF rat). Doses ranged from 0.2 to 15 mg/kg in mouse, rat and monkey studies. No differences were observed in pharmacokinetics values derived from male or female animals. AUC and Cmax were proportionate to dose. Half-life did not appear altered by dose. A summary table of pharmacokinetics studies as provided by the sponsor is found under appendix B. Serum levels of the fusion protein were determined by an ELISA with a lower limit of quantification of 0.3 ng/ml. Due to the development of anti-TNFR:Fc antibodies in some animals the actual levels of and pharmacokinetic endpoints may be underestimated due to interference by the antibody in the analytical assay. With repeated administration after sc administration of TNFR:Fc apparent decreases in Cmax, AUC, and t1/2 were observed suggesting the formation of antibodies. For example in cynomolgus monkeys given twice weekly repeat sc doses, Cmax and AUC decreased with repeated dosing at 1, 5 and 15 mg/kg when values were compared using day 1 and day 22

observations (see table below). The immunosuppressive effects of TNFR:Fc suppressed the development of antibodies and lack of change in the pharmacokinetics relative to the lower doses is evident in the findings.

Dose, mg/kg	Day 1 Cmax, ug/ml	Day 22 Cmax, ug/ml	Day 1 AUC, ug-hr/ml	Day 22 AUC, ug-hr/ml	Day 1 t1/2, hr	Day 22 t1/2, hr
1	5.8	0.6	373	7	77	10
5	34	15	2179	517	50	10
15	109	115	6677	6282	46	27

Table of pharmacokinetics values derived from monkeys with day 1 compared to day 22 after repeated dosing.

A pharmacokinetic comparison of TNFR:Fc made at Immunex, ——— and ——— ——— was performed. Groups of 4 female monkeys were given vehicle or 15 mg/kg of the product sc with injection twice weekly for 2 weeks. No differences were observed in Cmax or AUC on day 1 or day 12 of the study.

TNFR:Fc (5.0 mg/kg) had little effect on the pharmacokinetics of methotrexate (2.5 mg/kg) in female CD VAF rats; however, the pharmacokinetics of TNFR:Fc may be altered by co-administration of methotrexate. On day 11 but not on day 1, AUC values for TNFR:Fc were higher in rats administered the combination as versus the fusion protein alone. AUC was lower on day 11 as compared to day 1. A possible explanation for these findings is that methotrexate suppressed the development of antibodies to TNFR:Fc, but did not completely eliminate them. Hence, AUC values were less as compared to day 1, but elevated compared to a concurrent group given the same dose of the fusion protein. The presence of antibodies were not determined the pharmacokinetic study of methotrexate-TNFR:Fc interactions.

List of Toxicity Studies

1. Pharmacokinetic and Toxicity Study of TNFR:Fc via Subcutaneous and Intravenous Injection in Cynomolgus Monkeys.

2. Twenty-Eight Day Subcutaneous Toxicity Study in Monkeys

3. 26-Week Subcutaneous Toxicity Study in Cynomolgus Monkeys with a 4-Week Recovery - 3 month interim summary of preliminary unaudited data

4. Comparative 2-Week Subcutaneous Tolerability and Toxicokinetic Study in Female Cynomolgus Monkeys. GTR 3292

5. A 28-Day Toxicity Study of [redacted] Formulation of TNFR:Fc in the Cynomolgus Monkey

6. Pilot Multiple Dose (Twice weekly for 12 weeks) Subcutaneous Study of TNR-001 in Rats to Assess the Potential for Neutralizing Antibody Formation: Results Through Week 4

7. Pilot Multiple Dose (Twice weekly for 12 weeks) Subcutaneous Study of TNR-001 in CD-1 Mice to Assess the Potential for Neutralizing Antibody Formation: Results Through Week 4

8. Pilot Multiple Dose (Twice weekly for 12 weeks) Subcutaneous Study of TNR-001 in Rabbits to Assess the Potential for Neutralizing Antibody Formation

9. Subcutaneous Developmental Toxicity Dose Ranging Study in Rats

10. Subcutaneous Developmental Toxicity and Perinatal and Postnatal Toxicity Study in Rats

11. Subcutaneous Developmental Toxicity Dose Ranging Study in Rabbits

12. Subcutaneous Developmental Toxicity Study in Rabbits (Interim Report)

13. Mutagenicity Test with TNR-001 in the Salmonella-Escherichia coli/Mammalian Microsome Reverse Mutation Assay Preincubation Method with a Confirmation Assay

14. Mutagenicity Test on TNR-001 in the L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay

15. Mutagenicity Test on TNR-001 in a Chromosome Aberration Study in Human Whole Blood Lymphocytes with a Confirmatory Assay with Multiple Harvests

16. Mutagenicity Test on TNR-001 in an In Vitro Mouse Micronucleus Assay

Review of Studies

1. Pharmacokinetic and Toxicity Study of TNFR:Fc via Subcutaneous and Intravenous Injection in Cynomolgus Monkeys. Protocol 2423-126. Apr 1992.

The toxicity and pharmacokinetics of TNFR:Fc was investigated using cynomolgus monkeys. Monkeys were given iv injections for 3 consecutive days, followed by an 18-day recovery period, and when given sc injections for 20 consecutive days, followed by a 14-day recovery period. Pharmacokinetics were studied upon injection prior on day 3 prior to initiating consecutive dosing. Animals were allocated to study groups in accordance with the chart below.

Group	Dosage, mg/kg/day	Group size, M/F
1, control iv	0	2/2
2. control sc	0	3/3
3. low iv dose	1.5	3/3
4. high iv dose	15	3/3
5. low sc dose	0.2	3/3
6. high sc dose	2	3/3

Table of groups and doses for sc/iv study.

Two animals/sex/group in groups 1 to 3 were killed on day 7. One animal/sex from groups 2 and 3 were killed at the end of the 18 day recovery period (on day 24). Two animals/sex/group in groups 1, 4, 5 dosed sc were killed on day 24 following the dosing phase. The surviving group 1, 4, and 5 animals dosed by sc were killed on day 38 following a 14 day recovery period.

Endpoints included mortality, daily observations, ophthalmoscopic examinations, body weight, ECG, clinical pathology, gross and microscopic anatomy, clinical chemistry, hematology, urinalysis and coagulations parameters.

No biologically meaningful changes were found during the course of the study. Pharmacokinetic results were not reported.

2. Twenty-Eight Day Subcutaneous Toxicity Study in Monkeys. Lot 002755. Jun 1997. GTR-28856.

The purpose of this study was to determine the effects of 0, 1, 5, or 15 mg/kg/day given twice weekly and administered sc to cynomolgus monkeys (N = 3/sex/group) over a 28 day period. Various endpoints were measured: mortality, clinical observations, body weight, food consumption, ophthalmology, hematology, histopathology, clinical chemistry, urinalysis, serum drug levels, and antibody levels. Additionally, paraffin sections of kidneys from selected monkeys were stained with [redacted] and [redacted] to detect capillary thickening and the deposition of proteins as immune complexes. No adverse effects were observed which were related to the test article. However, a compound related effect on adrenal gland weight in females given 5 or 15 mg/kg. This effect was observed as an increase in mean organ weight whether it was determined as an absolute or adjusted by body weight. Changes in adrenal weight was not considered to be of toxicological significance as there were no changes in macroscopic, microscopic appearance or clinical pathology. Furthermore, changes in adrenal weight was not observed in male monkeys and the adrenal weights for females did not exceed the range of

historical controls for female animals.

Glomerulosclerosis occurred in the kidneys of 2 male monkeys given 1 mg/kg and 2 male monkeys given 5 mg/kg, as well as 1 female monkey given the vehicle and 2 female monkeys given 15 mg/kg. Glomerulosclerosis was unilateral and involved only 1 to 3 glomeruli in the sections examined.

Indices of exposure as Cmax or AUC increased in a linear manner by dose. A decrease in exposure occurred by day 22 relative to day 1 at doses of 1 and 15 mg/kg. AUC decreased by 98% and 77% and Cmax decreased by 90% and 55% . Plasma levels in monkeys given 15 mg/kg were not significantly lower on day 22 relative to day 1. Pharmacokinetics values are presented below. Antibodies were detected on day 15 in some animals and increased with continued dosing (see below).

Dosage, mg/kg	Cmax, ug/ml		AUC, ug-hr/ml	
	day 1	day 22	day 1	day 22
1	6	.6	373	7
5	34	15	2179	517
15	109	115	6677	6282

Table of mean values for pharmacokinetic parameters.

Dose, mg/kg	day 1 thru 12	day 15 & 19	day 22	day 26
0	0/6	0/6	0/6	0/6
1	0/6	6/6, 6/6	6/6	6/6
5	0/6	3/6, 6/6	6/6	6/6
15	0/6	0/6, 5/6	4/6	2/6

Table of antibodies detected during the toxicity study.

3. 26-Week Subcutaneous Toxicity Study in Cynomolgus Monkeys with a 4-Week Recovery - 3 month interim summary of preliminary unaudited data. GTR 32917.

This study will evaluate the chronic administration of TNFR:Fc in monkeys following twice weekly sc dosing. Monkeys will be dosed for 26 consecutive weeks and to determine the reversibility of any toxicity a 4 week recovery period was incorporated into the experimental design of the study. Doses are 0, 1, 5, or 15 mg/kg with 6 monkeys/sex/group. The interim report covers the first 3 month period of the study. The following endpoints were evaluated:

mortality, daily observations, ophthalmoscopic examination, ECG. body weight. food consumption, hematology, clinical chemistry, urinalysis, gross and microscopic anatomy. Sera will be evaluated for toxicokinetics. To date no test article related effects were observed in any endpoint. Thickened skin at the dosing site was observed for 1 female each in the 1 and 5 mg/kg group.

4. Comparative 2-Week Subcutaneous Tolerability and Toxicokinetic Study in Female Cynomolgus Monkeys. ——— lot 003821 and ——— lot 004687. Feb 1998. GTR 3292

This study compared the toxicokinetics and Tolerability of 2 lots of TNFR:Fc (TNR-001-B and TNR-001-T) which were manufactured at 2 different facilities (——— lot 003821 and ——— lot 004687). Female monkeys (N = 4) were given twice weekly sc injections for 2 weeks at 0 or 15 mg/kg/dose. Animals were doses on day 1, 5, 8, and 12 and killed on day 17. Endpoints included clinical observation, clinical chemistry, hematology and gross and microscopic examination of selected tissues. No toxicities were observed. No pharmacokinetic differences were found. Given the small number of animals incorporated into the study and the parallel design of the toxicokinetics study, only major differences in the pharmacology, toxicology or pharmacokinetics could possibly be detected.

5. A 28-Day Toxicity Study of ——— Formulation of TNFR:Fc in the Cynomolgus Monkey. Batch FXH0002A, FXH0002D, and FXH0002H. Protocol 90886. Apr 1996.

Two groups of cynomolgus monkeys (N = 3/sex/group) were exposed by inhalation (oronasal) to a ——— formulation at a ——— of either 0.29 (LD) or 0.81 (HD) mg/L TNFR:Fc for 16 minutes per day over a period of 28 days. Achieve doses were calculated to be 0.15 or 0.70 mg/kg/day of TNFR:Fc. A vehicle control was included in the study and had a ——— of 0.79 mg/L. Various observations were made during the course of the study which included: cage side observations, body weights, respiratory minute volume, hematology, clinical chemistry, urinalysis, pharmacokinetics, and antibody development. Two animals (1 from the LD and 1 from the HD groups) exhibited a slight increase in the number of granulocytic cells and increased M:E ration after dosing. Lung weights both absolute and relative were higher in the HD group (males reached statistical significance although females did not). The increases in lung weight were accompanied by perivascular cell infiltrates and intra-alveolar histiocytosis. Mononuclear cell infiltrates were observed in the lungs of 4 of 6 animals in the LD group and 6 of 6 monkeys in the HD group. Results of the pharmacokinetics and antibody portions of the study were not reported.

6. Pilot Multiple Dose (Twice weekly for 12 weeks) Subcutaneous Study of TNR-001 in Rats to Assess the Potential for Neutralizing Antibody Formation: Results Through Week 4. Lot 5939-019. GTR 2882[illegible]. Apr 1997.

This study was to investigate the extent and nature of antibody formation in female rats given twice weekly sc doses of 0, 1 or 25 mg/kg/dose (N = 6) for 12 weeks. Blood samples were taken at predose, 1, 2, 3, 4, 8, and 12 weeks after the start of dosing. By the first week of the study, 1 of 6 rats developed antibodies TNFR:Fc in the 1 mg/kg dosing group. By week 2, 1 of 6 rats developed antibodies in the 25 mg/kg dosing group. By the fourth week of the study all rats developed antibodies in the 1 mg/kg group and 9 of 12 in the 25 mg/kg group. Antibodies were found to neutralize the effect of TNFR:Fc. Antibody formation was assess by the [redacted] and [redacted].

7. Pilot Multiple Dose (Twice weekly for 12 weeks) Subcutaneous Study of TNR-001 in CD-1 Mice to Assess the Potential for Neutralizing Antibody Formation: Results Through Week 4. Lot. 5939-019. GTR 28822. Apr 1997

This study was to investigate the extent and nature of antibody formation in female CD-1 mice given twice weekly sc doses of 0, 1 or 25 mg/kg/dose (N = 5/time point/group) for 12 weeks. Blood samples were taken at predose, 1, 2, 3, 4, 8, and 12 weeks after the start of dosing. By the first week of the study, 1 of 5 mice developed antibodies TNFR:Fc in the 1 mg/kg dosing group. By week 2, 5 of 5 rats developed antibodies in the 1 mg/kg and 4 of 5 mice in the 25 mg/kg dosing groups. By the fourth week of the study all rats developed antibodies in the 1 mg/kg group and 9 of 12 in the 25 mg/kg group. Antibodies were found to neutralize the effect of TNFR:Fc. Antibody formation was assess by the [redacted] and [redacted]

8. Pilot Multiple Dose (Twice weekly for 12 weeks) Subcutaneous Study of TNR-001 in Rabbits to Assess the Potential for Neutralizing Antibody Formation. Lot 5939-019. GTR-28823. Apr 1997.

Three groups of female rabbits (N = 5/group) were given twice weekly TNFR:Fc sc at doses of 0, 1, or 25 mg/kg for 2 weeks. Blood samples were taken predose, and days 7, 10 and 14. Rabbits exhibited antibody development by day 10 in the 1 mg/kg group. By day 14, all rabbits in the 1 mg/kg and 3 of 5 rabbits using the [redacted] and 4 of 4 using the [redacted] n the 25 mg/kg group demonstrated antibodies.

9. Subcutaneous Developmental Toxicity Dose Ranging Study in Rats. Nov 1997. GTR 30354

Dosages of 0, 5, 15, or 50 mg/kg were given sc to CD VAF female rats. Both teratogenicity with N = 8 and pharmacokinetics with N = 28 were evaluated in the study. Doses were injected once per day from gestation day (GD) 6 through 17. Mortality, clinical observations, body weight, food consumption, gravid urterine weight, hysterotomy findings on GD 21 (corpora lutea, litter size, embryo/fetal mortality), postmortem findings, fetal gender, fetal weight, fetal gross external and palatal anomalies, and placental appearance were evaluated. Blood samples ere taken from a separate group of animals for toxicokinetic and antibody assessments on GD 6 to 7 and 17 to 18. No effects of TNF:Fc were observed on any toxicity endpoint. Accumulation of drug was

observed during its course of administration. Accumulation ratios of between 2.4 and 4.2 were observed and proportional between doses. Exposure increased with increasing dose after study days 1 and 12. For doses of 5, 15, and 50 mg/kg/day, the mean (±SE) of Cmax on study day 1 (GD 6) at 24 hours were 11.0 ± 1.8, 40.7 ± 3.3, and 99.4 ± 8.3 ug/ml; on study day 12, Cmax values were 26.3 ± 7.5, 68.9 ± 13.1 and 222 ± 8 ug/ml; AUC values were 448 ± 70, 1214 ± 114 and 4518 ± 369 ug-hr/ml. Accumulation did not appear dependent on dose as AUC/dose was comparable across all doses on day 12 (ie., 88, 81, 90). No antibodies were detected during the course of the study.

10. Subcutaneous Developmental Toxicity and Perinatal and Postnatal Toxicity Study in Rats. Mar 1998. Lot 004694. GTR 31008.

The developmental toxicity as well as perinatal and postnatal toxicity was studied in time mated female CD VAF rats (N = 25) from GD 6 through 20 for developmental toxicity and GD 6 through 21 for perinatal/postnatal toxicity at dosage of 0, 3, 10, or 30 mg/kg sc. For treated females, mortality, clinical observations, body weight, food consumption, gravid uterine weight, hysterotomy findings on GD 21 (corpora lutea, litter size, embryo/fetal mortality), fecundity parameters (gestation index, gestation length, parturition), maternal care of offspring for females and postmortem finding (including uterine implantation sites) were evaluated. Fetuses were examined for gender, weight, gross external and palatal anomalies, and visceral or skeletal anomalies. Placentae were examined for gross external appearance. Pups were examined for litter size, mortality, clinical observations, gender, milk-in-stomach, and body weight through PD 4. Daily sc injections to pregnant rats did not affect maternal gestation, parturition, and lactation and did not affect pre- and postnatal offspring development through PD 4 at dosages up to and including 30 mg/kg.

Serum exposure to TNFR:Fc increased proportionately with dose. Mean AUC's were on GD 15 159, 733 and 2026 ug-hr/ml. Means serum concentrations six hours after dosing on GD 15 and 20 were comparable which suggested a lack of neutralizing antibodies. Furthermore, a direct test for neutralizing antibodies failed to detect their presence in serum.

11. Subcutaneous Developmental Toxicity Dose Ranging Study in Rabbits. Lot 6025-025. Nov 1997. GTR 30392.

Mated female rabbits were used to evaluate the developmental toxicity of TNFR:Fc to offspring in terms of embryo/fetal mortality, fetal weight, and gross external morphology. Doses of 0, 5, 15 or 50 mg/kg were injected sc to groups of time-mated New Zealand White rabbits (N = 6). The following endpoints were measured: mortality, clinical observations, abortion rate, body weight, food consumption, gravid uterine weight, hysterotomy findings, fetal weight, fetal gross external and palatal anomalies, and placental appearance. Doses were given daily from gestation day 6 through 20. No adverse events occurred during the course of the study.

Pharmacokinetic indices of exposure in the 5 and 15 mg/kg groups decreased with dosing from

GD 6 through 20, an effect likely due to the development of neutralizing antibodies. On GD 20, 4 of 4 rabbits in the 5 mg/kg group and 1 of 4 in the 15 mg/kg group tested positive for neutralizing antibodies. No neutralizing antibodies were found in the 50 mg/kg group. On GD 6 mean AUC's were 582, 1437 and 4340 ug-hr/ml and on GD 20, levels were 6, 686 and 7406 ug-hr/ml for doses of 5, 15 and 50 mg/kg/day.

12. Subcutaneous Developmental Toxicity Study in Rabbits. Lot 004694. March 3, 1988. (GTR 30965) (Interim Report)

Doses of 0, 4, 13 or 40 mg/kg were given sc daily (GD 6 through 18) to time-mated New Zealand White (SPF) female rabbits (N=20). Mortality, clinical observations, abortion rate, body weight, food consumption, gravid uterine weight, hysterotomy findings on DG 29 (corpora lutea, litter size, embryo/fetal mortality) and postmortem findings were determined. Serum samples on GD 6, 15 to 16, and 18 were obtained from 4 animals at each dose. Fetuses were examined for gender, weight, and gross external appearance, palateal, visceral, and skeletal anomalies. Placenta were grossly examined. A slight to moderate increase in maternal food consumption (2% to 5%) and body weight gain (16% to 54%) as compared to the vehicle control occurred during the course of study in all groups. No adverse findings were identified with treatment during the period of dosing. Pharmacokinetics were not reported at this time.

13. Mutagenicity Test with TNR-001 in the Salmonella-Escherichia coli/Mammalian Microsome Reverse Mutation Assay Preincubation Method with a Confirmation Assay. Lot 001546. GTR 28291. Aug 1996.

The ability to induce reverse mutations was examined using the *Salmonella typhimurium* tester strains ——— and in the *Escherichia coli* tester strain ——— in the presence and absence of an exogenous rat liver microsome activation system —. No positive increase in the mean number of revertants per plate was observed up to 200 ug per ml.

14. Mutagenicity Test on TNR-001 in the ——— Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay. Lot 001546. Aug 1996. GTR 28297.

The ability to induce forward mutations was determined using the thymidine kinase (TK) locus in — mouse lymphoma cells in vitro in the absence and presence of — Up to 200 ug/ml, no increase the mutation frequency was observed under the conditions of the test.

15. Mutagenicity Test on TNR-001 in a Chromosome Aberration Study in Human Whole Blood Lymphocytes with a Confirmatory Assay with Multiple Harvests. Lot 001546. Aug 1996. GTR 28421.

The ability of induce chromosome aberrations was assessed in human whole blood lymphocytes

with and without metabolic activation (—. Up to 200 ug/ml, no significant increases were observed in the cell number with chromosomal aberrations.

16. Mutagenicity Test on TNR-001 in an In Vitro Mouse Micronucleus Assay. Lot 001546. Dec 1996. GTR 28832.

The ability to induce micronuclei was assessed in bone marrow polychromatic erythrocytes (PCE) of C rT:CD-1 *(ICR)BR mice. No significant increase in micronucleated PCE were observed with doses up to 200 mg/kg.

Conclusion

The pharmacology, pharmacokinetics and toxicology of TNFR:Fc was studied in a number of species including mice, rabbits, rats and monkeys using different routes of administration (subcutaneous, intravenous, and inhalation). TNFR:Fc was well tolerated in all species at doses representing approximately a 100 fold increase above clinical, therapeutic doses; alternatively, this may be expressed as a systemic equivalent which is approximately 74 fold greater than the anticipated human exposure. No adverse effects were observed following twice weekly sc dosing of up to 15 mg/kg for 28 days in monkeys (or an approximate 30 fold multiple above the therapeutic dose or pharmacokinetic exposure level). Neither developmental toxicities in rats or rabbit nor any genotoxicities were observed.

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